TAMPEREEN TEKNILLINEN YLIOPISTO
TAMPERE UNIVERSITY OF TECHNOLOGY

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EFFECTS OF ADDITIVE ADDITIONS ON UP-FLOW ANAEROBIC SLUDGE BLANKET REACTOR PERFORMANCE

Master of Science Thesis

Tarkastaja: professori Jukka Rintala
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Anaerobisella jätevedenpuhdistuksella tarkoitetaan menetelmiä, joissa mikro-organismit hajottavat jäteveden orgaanista ainesta hapettomissa olosuhteissa tuottaen energiarikasta biokaasua. Biokaasu koostuu pääosin metaanista ja hiilidioksidista ja sitä voidaan käyttää energianlähteenä sähkön- ja lämmöntuotannossa. Ylöspäinvirtaus lietepatja-reaktori (UASB) on yksi käytetyimmistä anaerobisista jätevedenpuhdistusmenetelmistä. Reaktorin sisällä oleva lietepatja koostuu granuloista, jotka ovat mikro-organismien muodostamia aggregaatteja.

Mikro-organismit tarvitsevat hivenaineita ylläpitääkseen niille tärkeitä elintoimintoja. Laboratoriotutkimuksissa hivenainelisäyksillä on saavutettu positiivisia vaikutuksia UASB –reaktorin toimintaan. Tämän työn tavoite oli tutkia hivenainepohjaisten lisäaineliuoksien vaikutusta UASB -reaktorin kuormitettavuuteen. Viittä UASB reaktoria operoitiin panimojätevedellä 83 päivää. Yksi reaktori oli kontrolli, neljään muuhun lisättiin lisäaineliuos: BDP-liuos (sisälsi Fe, Ni, Co, Se ja Mo), liuos A (sisälsi BDP-881 ja Zn), liuos B (sisälsi BDP-881 sekä Zn ja Mn) ja liuos C (sisälsi BDP-881 sekä Zn, Mn ja Ca).

Kontrollireaktorin metaanintuotto väheni merkittävästi kuormituksen ollessa 14 g SCOD/l/d (koepäivä 64). Kuormituksella 16 g SCOD/l/d (koepäivä 75) metaanintuotto keskeytyi. Samalla kuormituksella hivenainesyöttöiset reaktorit toimivat ongelmitta. Hivenainelisäys mahdollisti 37 % korkeamman kuormituksen verrattuna kontrollireaktoriin. Kun metaanintuotto oli keskeytynyt kontrollireaktorissa, sen syötteeseen lisättiin myös BDP-881 -liuosta. Muutamassa päivässä reaktori elpyi ja metaanintuotto sekä COD vähenemä nousivat 0:sta 1,7:än l/d ja 7:stä 43:en %.

Tutkimuksen tulosten perusteella on todennäköistä, että BDP-881 yksinään ehostaa reaktorin toimintaa, sillä hivenainesyöttöisten reaktoreiden toiminnassa ei ollut merkittäviä eroja. Eroja olisi kuitenkin voitu havaita jos kuormitusta olisi nostettu entisestään. Maksimikuormitus hivenainesyöttöisille reaktoreille tulisi määrittää, ennen kuin liuoksia käytetään teollisissa sovelluksissa.

ABSTRACT

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In anaerobic wastewater treatment micro-organisms convert the organic compounds in the wastewater in the absence of oxygen and produce energy-rich biogas. Biogas consists mainly of methane and carbon dioxide and it may be used in electricity and heat production. Up-flow anaerobic sludge bed reactor (UASB) is one of the most used anaerobic wastewater treatment technologies. Sludge bed inside the reactor consist of microbial aggregates called granules.

Micro-organisms require trace elements to sustain their vital functions. Laboratory experiments have confirmed the positive effects of trace element additions in the UASB reactor's performance. The objective of this experiment was to study the effects of added trace element based nutrient solutions on brewery wastewater fed UASB reactor's performance. Five UASB reactors were operated for 83 days. One reactor was kept as a control reactor, and the other four were fed with nutrient solutions: Solution BDP-881 (containing Fe, Ni, Co, Se, and Mo), Solution A (containing BDP-881 and Zn), Solution B (containing BDP-881, Zn and Mn) and Solution C (containing BDP-881, Zn, Mn and Ca).

The methane production in the control reactor dropped significantly, when organic loading rate (OLR) was 14 g SCOD/l/d (operation day 64). At OLR 16 g SCOD/l/d (operation day 75) the methane production halted. At the same OLR the trace element fed reactors kept working properly. Trace element addition allowed 37 % higher OLR compared to the control reactor. After the methane production halted in the control reactor, it was also fed with BDP-881 solution. In a couple of days, the reactor recovered and methane production and COD removal rose from 0 l/d to 1.7 l/d and from 7 % to 43 %, respectively.

According to this study, it is likely that BDP-881 alone improves the reactor performance, because there were no differences in reactor performance between four nutrient solutions fed reactors. However, there could have been differences, if OLR would have been elevated even more. Maximum OLR for trace element fed reactors should be determined, before the solutions are used in industrial applications.

PREFACE

This experiment was carried out in Tampere University of Technology in the department of chemistry and biotechnology under supervision of Professor Jukka Rintala.

I would like to thank my supervisor Jukka for the opportunity to work in this project and for his advices and comments during the experiment. I am grateful to scientists Sakari Halttunen, Kaisa Karisalmi, and Saku Liuksia from Kemira, who gave me lots of advices during the experiment.

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Tampere, XXth of xxxber 2013.

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HENRI HÄSÄ

TABLE OF CONTENT

NOMENCLATURE

UASB	Up-flow anaerobic sludge bed
HRT	Hydraulic retention time
OLR	Organic loading rate
COD	Chemical oxygen demand
SCOD	Soluble chemical oxygen demand
VFA	Volatile fatty acids
TS	Total solids
VS	Volatile solids
VSS	Volatile suspended solids
OHPA	Obligate hydrogen producing acetogens
AMA	Acetotrophic methanogenic archae
HMA	Hydrogenotrophic methanogenic archae
EPS	Extracellular polymeric substance
SMA	Specific methanogenic activity
CH_4	Methane
CO_2	Carbon dioxide
H_2	Hydrogen
$CaCO_3$	Calcium carbonate
$CaHPO_4$	Calcium hydrogen phosphate
CO	Carbon monoxide
HNO_3	Nitric acid
CODH	Carbon monoxide dehydrogenase

1 INTRODUCTION

Anaerobic wastewater treatment is a favorable form of wastewater treatment application. First of all, it is suitably for high strength wastewater. Secondly, because no oxygen is needed the energy requirements are low. Finally, the produced methane (CH_4) through anaerobic conversion may be used as renewable energy (Seghezzo et al. 1998). Industrial anaerobic wastewater treatment applications are used in breweries, distilleries, food-, beverages-, and fermentation industry, to name a few (Tchobanoglous et al. 2003). One common anaerobic wastewater treatment application is called an up-flow anaerobic sludge blanket (UASB) reactor (Liu and Tay 2004).

UASB reactor's core is the granular sludge bed at the bottom of the reactor tank. This sludge bed consists of microbial aggregates (also called granules) which use the substrates in the wastewater fed into the reactor. These granules are very dense and their diameter ranges generally from 0.5 to 2.0 mm. Due to the large size of the granules they resist washout, which allows sustaining high hydraulic loads in UASB reactor. This is one of the biggest advantages compared to other reactors. The granulation process is complicated and still not fully discovered how it occurs step by step. There are several theories considering the granulation process, but none of them have been proven to be exactly true. (Liu et al. 2002)

It has been studied that laboratory UASB reactor's performance may be improved with the addition of trace elements into the feed of the reactor. In biochemistry, trace elements are needed in very small quantities for the proper growth, development and physiology of organisms (Bowen, 1976). They usually act as parts of the active site of different microbial enzymes (Oleszkiewicz and Sharma 1990; Zandvoort et al. 2006). Several studies suggest that trace element addition has effects on UASB reactor's performance. Increased chemical oxygen demand (COD) removal and CH_4 production have been reported in recent studies considering trace element addition into the feed of a UASB reactor (Fermoso et al. 2008). However, addition of trace elements doesn't always have positive effects. Inhibition may occur, when concentrations for one (Alkan et al. 1995; Bhattacharya et al. 1995; Lin and Chen 1999) or several (Ram et al. 2000; Fermoso et al. 2008; Atlas 2009) elements elevates too high.

The objective of this study was to find out the effects of trace elements on UASB reactor's performance treating brewery wastewater with increasing organic loading rates (OLR). Effects of added trace elements were studied in five brewery wastewater fed

UASB reactors. One reactor was kept as a control reactor and fed only with the brewery wastewater, whereas the four other reactors were fed with brewery wastewater containing different trace element based nutrient solutions.

2 THEORETICAL BACKGROUND

2.1 Anaerobic conversion

Anaerobic conversion is a natural process where organic material is broken down by micro-organisms in the absence of oxygen. This organic matter may consist of food waste, slurry, manure, domestic or industrial wastewater, crop or crop residues, for instance. When the organic matter is digested, methane-rich biogas is released. This gas consists of CH_4 (approximately 60 v-%), carbon dioxide (CO_2) (approximately 40 v-%), and traces of other gases. The exact composition depends highly on the composition of the conversed organic matter. (Maria et al. 2012)

Organic matter is complex and the anaerobic conversion has several steps involving metabolic reactions before the organic matter is finally converted into CH_4 (Mata-Alvarez 2002). The important intermediaries of CH_4 formation have been identified and the overall view of the anaerobic conversion is depicted in Figure 2.1.

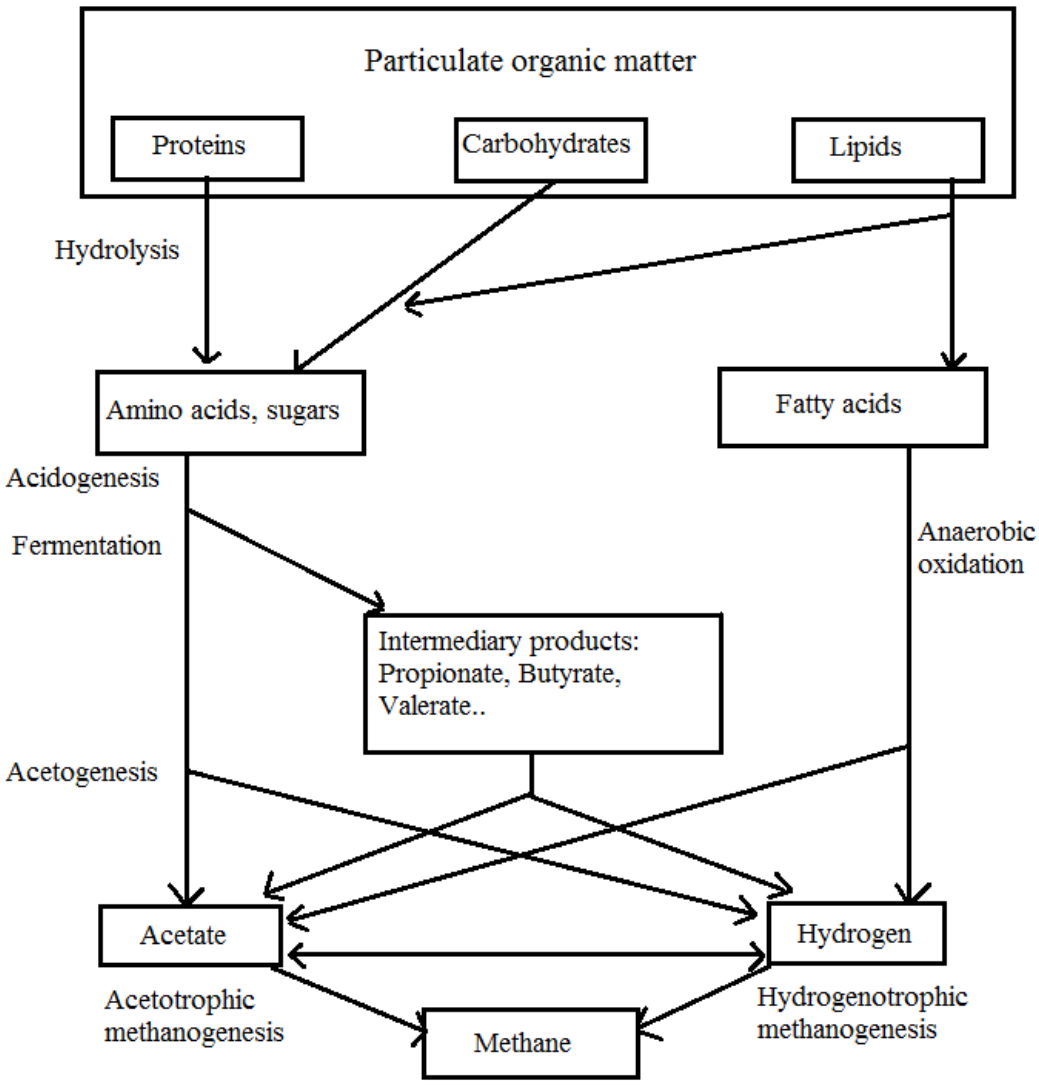


Figure 2.1. *Scheme of the biodegradation steps of complex organic matter, modified (Siegrist et al. 1993)*

Anaerobic conversion occurs in synergy of several species of different micro-organisms. The conversion has four different phases, in which different microbial populations are active. The phases are hydrolysis, acidogenesis, acetogenesis and methanogenesis (acetotrophic and hydrogenotrophic). (Mata-Alvarez 2002)

During hydrolysis the complex organic matter consisted of carbohydrates, lipids, and proteins is disintegrated to short chain carbohydrates, long chain fatty acids, and amino acids. Hydrolytic bacteria have the ability to produce hydrolytic enzymes, which degrade both insoluble and soluble high-molecular weight organic compounds (Mata-Alvarez 2002). During acidogenesis fermentative bacteria degrade the organic monomers of sugars and amino acids and produce hydrogen (H_2), CO_2, acetate and other volatile fatty acids (VFA), such as propionate, butyrate, and valerate. Ammonia is also

produced by the degradation of amino acids (Mata-Alvarez 2002). During acetogenesis long chain fatty acids and VFAs are converted into acetate, CO_2 and H_2 by obligate hydrogen producing acetogens (OHPA) (Mata-Alvarez 2002).

The final stage of anaerobic conversion is called methanogenesis, where the compounds are converted into CH_4. CH_4 is formed by hydrogenotrophic methanogenic archae (HMA) and acetotrophic methanogenic archae (AMA). HMA produce CH_4 from H_2 and CO_2 by the hydrogen-consuming archae in a syntrophic co-culture with the OHPA, whereas AMA produce CH_4 and CO_2 from acetate. HMA work faster than AMA, but the acetotrophic methanogenesis accounts for the most of the CH_4 produced in the overall process. (Mata-Alvarez 2002)

A requirement for properly occurring anaerobic conversion is that the microbial populations are balanced. Methane-formers have much slower growth rate compared to acidogenic bacteria. If the acid-forming micro-organisms outgrow methane-formers acidic conditions becomes prevalent. This may slow down the activity of methanogens and eventually totally inhibit their activity (Mata-Alvarez 2002). To prevent process failure in practical applications, a proper monitoring and control of temperature, alkalinity, pH, VFA, and nutrients are needed.

Micro-organisms have different temperature ranges, where their vital functions work optimally. Steady temperature during the anaerobic conversion is often more favorable for micro-organisms than variable temperature. Two optimal temperature ranges with maximum activity have been identified: mesophilic (approximately 35 ^{o}C) and thermophilic (approximately 55 ^{o}C) (Hernon et al. 2006). Mesophilic range is more robust and more stable environment, but thermophilic range provides better CH_4 production and pathogen extermination (Mata-Alvarez 2002).

High concentrations of VFA, free ammonia and hydrogen sulphur may also lead to the inhibition of anaerobic conversion. As mentioned, VFAs are intermediary compounds of the anaerobic degradation of organic matter. pH and alkalinity level exerts a definite effect on VFA toxicity, and the threshold level depends on these parameters. Propionic and butyric acids have been described as the most inhibitory among VFAs. Inhibitory concentration of free ammonia and hydrogen sulfide also depends on different parameters, such as pH and alkalinity. (Mata-Alvarez 2002; Lindeboom et al. 2013)

2.2 Anaerobic wastewater treatment

Anaerobic wastewater treatment is a form of biological wastewater treatment technology in the absence of oxygen. Micro-organisms use the compounds in the wastewater, which enhances the quality of the wastewater. Simultaneously, the compounds are converted into biogas through the anaerobic conversion. The biogas contains CH_4, which

makes the gas energy rich, and it may be used in heat and electricity production. Anaerobic wastewater treatment is especially suitable for wastewater with high concentration (COD concentration over 4 g/l). (Chan et al. 2009)

Compared to aerobic wastewater treatment, anaerobic systems have some remarkable advantages. First of all, high OLR is possible with lower energy and nutrient requirements. Also, amount of produced sludge is low. However, a major drawback in anaerobic wastewater treatment system is its relatively long start up time, which is usually several months. Also, anaerobic wastewater treatment is considered more sensitive to temperature changes, compared to aerobic systems. Table 2.1 shows the comparison for aerobic and anaerobic wastewater treatment. (Chan et al. 2009)

Table 2.1. *Comparison of aerobic and anaerobic wastewater treatment (Chan et al. 2009)*

Feature	Aerobic	Anaerobic
OLR	Moderate	High
Sludge production	High	Low
Nutrient requirement	High	Low
Energy requirement	High	Low to moderate
Startup time	2-4 weeks	2-4 months
Temperature sensitivity	Low	High

In practical wastewater treatment applications anaerobic systems don't provide a complete stabilization of the organic matter in the wastewater. Also, effluent contains usually ammonium ions and hydrogen sulfides (Heijnen et al. 1991). To achieve a sufficient quality for treated wastewater, the effluent from anaerobic system is treated aerobically afterwards. The effluent produced by the anaerobic treatment contains solubilized organic matter, which is suitable for aerobic treatment (Gray et al. 2005). Anaerobic or aerobic wastewater treatment doesn't provide efficient wastewater treatment alone, but used together they make very favorable and efficient applications (Aggelis et al. 2001).

2.3 UASB reactor

A UASB reactor consists of a large tank, where wastewater is fed from the bottom and removed from the top. At the bottom of the reactor there is a bed of granular sludge, which consists of microbial aggregates. As the wastewater passes through the sludge bed, the micro-organisms use the wastewater as a substrate and produce biogas. Released biogas is separated from the wastewater and collected with baffles and a gas cap from the top of the reactor. Biogas production causes hydraulic turbulence, which causes the granular sludge to mix without any mechanical parts. Figure 2.3 depicts the concept of a UASB reactor. (Seghezzo et al. 1998)

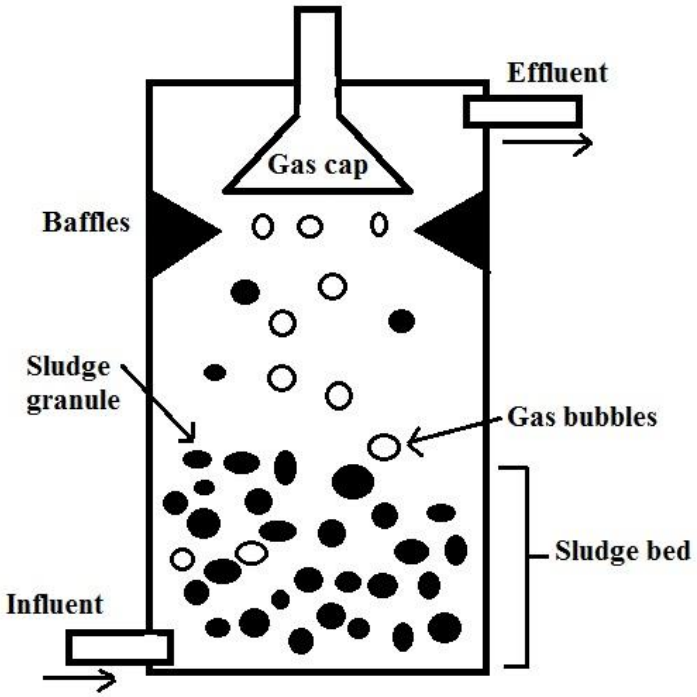


Figure 2.2. *The upward-flow anaerobic sludge bed reactor concept, modified (Liu et al. 2003)*

The core of UASB reactors are the sludge granules at the bottom of the tank. A granule is an aggregate of micro-organisms. When there isn't any support matrix present and the flow conditions are favorable, the micro-organisms start to attach to each other. This way the micro-organisms are able to survive and proliferate, and eventually the aggregates form into a dense compact granule. Granule's diameter generally ranges from 0.5 to 2 mm. Due to the large size of granules, they resist washout from the reactor, which allows sustaining high hydraulic loads. Granules are dense and the concentration of active micro-organisms is high, which provides high CH_4 production. (Vlyssides et al. 2008)

Granulation process is a complicated process and it usually takes several months to form applicable sludge bed for a full-size UASB reactor. There are several theories considering the granulation process. Conclusions have been drawn about the triggering forces and steps occurring in the process. Liu et al. (2002) have proposed a four step general model for granulation process, which is gathered from other theories. The model proposes that chemical, physical, and biological forces cannot be considered separately but in synergy in the granule formation. The model also proposes that there are four main steps in the granulation process.

During the first step physical movement initiates bacterium-to-bacterium or bacterium-to-nuclei attachments and forces such as hydrodynamic forces, gravity forces, diffusion

forces, thermodynamic forces and cell mobility are involved. During the second step several physical, chemical, and biological forces makes the multicellular contacts stable. Forces such as hydrophobicity and cellular surface dehydration and membrane fusion have been emphasized to play a crucial role in the initiation of anaerobic granulation. During the third step microbial forces make the cell aggregation mature. Production of extracellular polymer and growth of cellular cluster occur. Finally, during the fourth step the three-dimensional structure of granules is shaped by hydrodynamic shear forces and structured communities are formed. The outer shape and size of microbial aggregates are determined by the interactive strength/pattern between aggregates and of hydrodynamic shear force, microbial species, and substrate loading rate. (Liu et al. 2002)

2.4 Trace elements

In biochemistry, trace elements are needed in very small quantities for the proper growth, development, and physiology of organisms (Bowen, 1976). Several studies suggest that trace element addition has effects on UASB reactor's performance. Increased COD removal and CH_4 production have been reported in many recent studies considering trace element addition into the feed of a UASB reactor (Fermoso et al. 2008). However, addition of trace elements doesn't always have positive effects. Inhibition may occur, when concentrations for one (Alkan et al. 1995; Bhattacharya et al. 1995; Lin and Chen 1999) or several (Ram et al. 2000; Fermoso et al. 2008; Atlas 2009) elements elevates too high.

Whether the effects are positive or negative depends highly on the role of added trace elements in biological processes. Trace elements are parts of the active site of enzymes, which makes them essential for micro-organisms (Oleszkiewicz and Sharma 1990; Zandvoort et al. 2006). Heavy metals act as inhibitors by blocking enzyme functions. This type of inhibition is characterized by the reversible binding of the inhibitor with either the enzyme or the enzyme-substrate complex. Metals may also act as competitive inhibitors for the substrate. This type of inhibition depends on the affinity of the metal and the enzyme, as well as on the relative concentrations of the competing metals (Oleszkiewicz and Sharma 1990).

2.4.1 Trace element interactions

The transport of metal ions is to a great extent generally determined by the properties of the transport systems (Braun et al. 1998). The uptake of metal ions by specific transporters can be described by Michaelis Menten kinetics: The bioavailable metal ion is first bound by a transporter site and subsequently taken up. The binding properties determine the affinity of the transporter to the metal, while the amount of the transporter determines the maximum uptake rate. Both of the mentioned parameters can change with changing chemistry and biology (Fermoso et al. 2008). Different metal ions can compete for the same uptake site, thus affecting the conditional affinity (Sunda and

Huntsman 1998). Also, micro-organisms can actively decrease or increase the number of the transporters in response to its environment, which affects to the maximum uptake rate (Worms et al. 2006).

Metal sorption in granules occurs due to precipitation, co-precipitation, adsorption, and binding by extracellular polymeric substance (EPS) and bacterial cells. EPS are major components of granular matrix, and up to 90 % of the dry biomass is EPS material (Gao et al. 2008), which bind metals (Guibaud et al. 2008). Also, the bacterial interface can act as a metal binding surface (Aksu et al. 1991).

The bioavailability and mobility of essential trace elements in the UASB reactors are mainly controlled by sulfide chemistry and the existence of copper, iron, zinc, and nickel sulfide precipitates in UASB granules have been confirmed with X-ray analyses (Fang and Liu 1995; Liu and Fang 1998; Gonzalez-Gil et al. 2001; Kaksonen et al. 2003; Van der Veen et al. 2007). Metal ions are expected to precipitate with sulfide, carbonate and phosphate in the pore water present in the granular matrix (Martell and Smith 1989). Metal sulfide precipitation is expected to be the most important process. The predominating role of sulfides in metal fixation in anaerobic granules in supported by the high acid volatile sulfide content and the high metal content in the oxidizable (containing both sulfidic and organic bonding forms) fraction present in UASB systems (Van der Veen et al. 2007).

Metal sulfides have a low solubility product and it would be expected that these metals are not bioavailable to the methanogenic consortia (Martell and Smith 1989). Ageing of sulfidic precipitates occurring in the sludge during reactor operation lowers the dissolution rates and may therefore lower the metal bioavailability (Gonzales-Gil et al. 2003). However, in most cases the dissolution rates of cobalt and nickel sulfides do not limit the methanogenic activity in anaerobic wastewater treatment (Jansen et al. 2007).

Metal sulfide precipitation is expected to be the most important process in metal precipitation in the anaerobic media present in the UASB reactor. The low solubility product of metal sulfides results in low free metal ion concentrations (Martell and Smith 1989). However, free metal ion concentrations from these solubility products should be predicted carefully, because there is couple of factors affecting to the concentration. First of all, the crystal structure of the metal sulfide precipitate should be known to find out the solubility product. Secondly, the concentration values in literature vary greatly. Thirdly, precipitation equilibrium is not reached in many cases due to kinetic limitations. Finally, size and ligand effects may affect to the precipitation (Fermoso et al. 2008).

Sulfide is important because of the formation of metal precipitates, but also because of the formation of dissolved metal complexes. Other important inorganic ligands are car-

bonate and phosphate (Bartacek et al. 2008). Carbonate is important, because of its high concentration in wastewaters and its strong binding with metal ions. Organic and synthetic ligands may also affect to metal precipitation, but interactions are usually weaker compared to sulfide (Martell and Smith 1989). Organic ligands may increase the concentration of dissolved metal or affect the size and kinetics of precipitation (Adams and Kramer 1998). Synthetic ligands in some cases keep the metals in wastewater dissolved, which affects to the bioavailability (Bretler and Marison 1996).

Metal precipitation can be divided into five stages: nucleation, growth of nuclei, aggregation, formation of irreversible aggregates and formation of larger crystals at the expense of smaller ones (Nielsen 1964). Precipitates age by generally transforming from amorphous precipitates to more stable crystalline forms.

The kinetics of precipitation and dissolution are influenced by organic ligands: they can decrease the precipitation rate (Helz and Horzempa 1983; Shea and Helz 1987) or increase the dissolution rate, for example in case of siderophores (Kraemer and Hering 1997; Liang et al. 2000; Cervini-Silva and Sposito 2002). Besides the reaction kinetics involving the particulate matrix, the rates of processes within the dissolved metal fraction can be important. In aqueous solutions, the rate of metal complex formation largely depends on the water loss rate constants of the metal ions (Morel, 1983), independent of the nature of the ligand.

2.4.2 Trace elements in UASB reactors

Due to the complexity of bioprocesses and differences between the characteristics in reactor influent, it is difficult to determine optimal concentrations for trace elements, even though the functions of different elements are known in biological processes (Liang et al. 2007). It is also difficult to draw a conclusion whether the elements affect in reactor performance individually or together. Nevertheless, results have been made in experimental studies, and plenty of information is available from the effects of added trace elements. Results from studies considering the addition of trace elements have been gathered in table 2.2 and effects of individual and several trace elements on UASB reactor's performance are presented in the following section.

Table 2.2. *Study results from additive additions in UASB reactors.*

Element	Reactor/method	Wastewater	t (°C)	Effect	Reference
Mg	UASB reactors fed with different concentrations of Mg2+ (0–2430 mg/l).	Acetate	55	Mg2+ concentration of 2430 mg/l caused disaggregation of Methanosarcina packets, release of a high number of single cells, corresponding to 20% of the biomass. In the absence of Mg2+, a decrease in the conversion of acetate was observed and 50% of the biomass was washed out from the reactor.	Schmidt and Ahring (1993)
Na	Different tests considering the effects of Na addition in UASB reactor performance.	Several	n.a.	Stable conditions in Na concentrations of 100-200, 230, and 350 mg/l. Inhibitory effects in Na concentration of 3.5 g/l and above.	Chen et al. (2007)
K	Different tests considering the effects of K addition in UASB reactor performance	n.a.	35 & 55	400 mg/l or less of K resulted in enhancement in performance in both the thermophilic and mesophilic ranges while at higher concentrations there was an inhibitory effect.	Chen et al. (2007)
Ca	Six UASB reactors fed with different Ca concentrations (0, 150, 300, 450, 600, 800 mg/l)	Synthetic wastewater (4 g-COD/l)	35	Ca2+ concentrations from 150 to 300 mg/l enhanced the biomass accumulation and granulation process. The specific activity of granules decreased with increasing influent Ca2+ concentration.	Yu et al. (2001)
Fe	Two UASB reactors were operated 1.4 to 10.0 g COD/l/d of OLR. Ferrous iron was fed only to other reactor in a range of load from 0.014 to 0.100 g Fe2+/l/d.	Synthetic milk wastewater (15-110 g-COD/l)	35	The addition induced a stable and increased COD conversion rate, increaced granule diameter and characteristics settling velocities compared to control reactor.	Vlyssides et al. (2008)
Fe	One UASB reactor was tested with different batch assays. Sulfide batch assays were done before Fe addition.	Ethanol (1 g-COD/l)	25 (± 2)	The addition of Fe, up to a 8.1 mM increased 40% the substrate utilization value compared to the rate obtained without metal addition (0.25 g COD/g Volatile suspended solids (VSS)-d). Fe concentration of 8.5 mM inhibited the specific substrate utilization rate by 57% compared to the rate obtained in the batch amended with 4.0 mM Fe2+ (0.44 g COD/g VSS-d).	Gonzales-Silva et al. (2009)

Element	Reactor/method	Wastewater	t (°C)	Effect	Reference
Zn	Two UASB reactors operated simultaneously. One was supplemented with different metals, the control was not.	VFA (acetate)	30	Specific methanogenic activity (SMA) increased by 36% compared to the control reactor.	Osuna et al. (2002)
Zn	Granular sludge was from UASB reactor, but actual experiments were done in batch bottles	Winery wastewater	35	50% inhibition of methanogenic activity with Zn concentration of 690 mg/l (HRT 1 d) and 270 mg/l (HRT 2 d).	Lin and Chen (1999)
Zn	Granular sludge was from UASB reactor, but actual experiments were done in batch bottles	Starch synthetic wastewater	37	50% inhibition of methanogenic activity with Zn concentration of 96 mg/l	Fang (1997)
Co	Two UASB reactors were operated with and without supplementation of cobalt.	Methanol	30	Increase COD removal, and 3 times higher methane productivity in the Co supplemented reactor compared to the control reactor.	Florencio et al. (1993)
Co	Anaerobic toxicity bioassays, performed with 150 ml serum bottles using acetate utilizing methanogenic enrichment culture.	Glucose	35 (± 1)	950 mg/l of added cobalt led to 100 % inhibition of methanogenic activity	Bhattacharya et al. (1995)
Co	Two UASB reactors supplemented with cobalt, one pre-loaded in the influent and the other dosed directly into the reactor.	Methanol	30	Increase SMA (5.8 times higher for preloaded, 4 times higher for in situ) and methanol removal.	Zandvoort et al. (2004)
Cr	Four bioreactors where used, first one was a control, second was dosed in a stepwise manner with increasing concentrations of Cr and the third and fourth were used for shock injections of Cr.	Synthetic feed (sweet whey powder)	30	500 mg/l injection of Cr led to process failure, whereas 400 mg/l led to strong inhibition.	Alkan et al. (1995)

Element	Reactor/method	Wastewater	t (°C)	Effect	Reference
Mn	Two UASB reactors operated simultaneously. One was supplemented with Mn, the other was a control reactor	VFA (acetate)	30	Conversion rate (mg COD/g VS d) of acetate and propiante increased 26 % and 260 %, respectively with Mn addition.	Osuna et al. (2002)
Ni	Experiments were performed in 100 ml serum vials by a batch test. Ni concentration range tested was 1 – 3000 mg/l	Winery Wastewater	35	Ni concentrations of 81 mg/l and above led to 50 % inhibition of VFA degradation.	Lin and Chen (1999)
Ni, Co & Fe	Three UASB reactors. R1 unadulterated; R2 supplemented with Ca and PO4; R3 supplemented with ferric chloride and traces of Ni and Co.	Food industry wastewater	35	Faster sludge growth and better sludge retention in R3 reactor compared to R2 and R1.	Oleszkkiewicz and Romanek (1989)
Ni, Co & Fe	Five UASB reactors. Granules in reactors were operated at the following conditions: (1) fed with VFAs supplemented with yeast extract for over 200 days, (2) the same feed, but without yeast extract, for 60 days and (3) no feeding but upflow liquid recirculation for 30 days. Feed for each reactor was supplemented with different concentrations of Ni, Co and Fe.	VFA	35	Supplements of Ni, Co and Fe did not influence the COD conversion in the reactors for 200 days. Yeast extract elimination decreased COD conversion after 60 days in Fe supplemented reactors. Most of Fe and Co in extracted EPS were found in bound form, which may be important in bacterial aggregation.	Shen et al. (1993)

Element	Reactor/method	Wastewater	t (°C)	Effect	Reference
W, Mo & Se	One UASB, which was first run without additives, then with several trace elements without W, Mo & Se. Third run included all trace elements and the last W, Mo & Se solely.	VFA	30 (± 2)	During a long-term absence of Mo, W and Se from the feed to the UASB reactor, the methanogenic activity decreased.	Worm et al. (2009)
Fe, Ni, Co & Mo	One UASB reactor was operated at OLR of 5-21.5 kg COD/m3 d. With an OLR of 17.4 kg COD/m3 d, an accumulation of VFA, principally propionic acid, was observed due to lack of trace metals (Fe, Ni, Co and Mo).	Cane molasses stillage	35	The addition of Fe (100 mg/l), Ni (15 mg/l), Co (10 mg/l) and Mo (0.2 mg/l) reduced the level of propionic acid (5291mg/l to 251 mg/l) and acetic acid (1100 mg/l to 158 mg/l). The COD removal efficiency increased from 44% to 58%, the biogas production from 10.7 to 14.8 l/d (NTP) and 0.085 to 0.32 g CH4-COD/g SSV d for specific sludge methanogenic activity with propionic acid as substrate. Results were obtained with high COD (68.9 g/l) and OLR (21.5 kg COD/m3 d).	Espinosa et al. (1995)

2.4.2.1 Iron

Iron is a necessary trace element for synthesis of various anaerobic organisms by acting as a co-factor for different enzymes. It acts as a hydrogenase, carbon monoxide (CO) dehydrogenase, nitric oxide reductace, and nitrite and nitrate reductase in anaerobic reactions (Fermoso et al. 2008). When supplemented as ferrous iron with 0.01 g Fe_{2+}/g COD feed dosing, it has had positive effects on synthetic milk wastewater fed UASB reactor's performance by increasing COD removal and granule diameter (Vlyssides et al. 2008).

Inhibitory effects of iron on reactor performance has also been tested for synthetic wastewater by adding iron as $FeCl_2$ $4H_2O$. Negative effects, such as decreased substrate utilization value, occurred when 140 ml shots in a batch experiment had iron concentration of 0.475 g/l or above. At the concentration of 0.452 g/l or below the utilization rate increased (Gonzales-Silva et al. 2009).

2.4.2.2 Calcium

Calcium has a positive effect on the flocculation ability of the anaerobic sludge. Calcium is required for several strains of *Methanosarcina* including *M. barkeri* and for the desegregation of individual cells in *M. mazei*. Present in the sheaths of *Methanospirillum hungatei*, calcium is the major cation. The surface of developed granules in the reactor supplemented with calcium and phosphate contained a significant number of *Methanothrix soehngenii*. Addition of calcium have densified and stabilized the granular sludge and resulted into a minimum wash-out. Also, the concentration of trace metals differed widely in the granular sludges grown under different calcium and phosphate conditions. (Singh et al. 1998 review)

Heavy precipitation of calcium carbonate ($CaCO_3$) and calcium hydrogen phosphate ($CaHPO_4$) in the sludge may be caused by higher concentration of calcium (Lettinga et al. 1991). Both hydrophobicity and the charge on surfaces are important as alterations in surface charge by removal of calcium ions (Ca^{2+}) may lead to a decrease in the granule strength and in some cases a complete disintegration of granules may result, which makes the precipitation of $CaCO_3$ and $CaHPO_4$ unfavorable for granulation (Wu et al. 1987). In UASB reactors, Ca^{2+} concentrations from 100 to 200 mg/l have been reported to be beneficial for sludge granulation, whereas Ca^{2+} concentrations greater than 300 mg/l were reported to be detrimental (Chen et al. 2007).

2.4.2.3 Cobalt

Cobalt is an essential trace element in anaerobic reactions and is one of the most studied trace elements for anaerobic reactors. In biochemistry, it acts as a core of B12-enzyme, a CO-dehydrogenase, and a methyltransferase (Fermoso et al. 2008). Pulse addition of cobalt in the form of cobalt chloride ($CoCl_2$) (0.64 mg/l) has been discovered to be

enough to maintain stable methanogenesis in UASB reactors at an OLR of 8.5 g COD/l/d (Fermoso et al. 2008). Sole supplementation of cobalt in methanol fed UASB reactors has also led to the increase of COD removal (Shen et al. 1993), increase of SMA and methanol removal (Zandvoorth et al. 2004), and high activity of methanogens (Paulo et al. 2004). Cobalt supplementation with nickel and iron has also had positive effects on UASB reactor performance, such as faster sludge growth and better sludge retention (Oleszkiewicz and Romanek 1989), and increased COD removal (Shen et al. 1993).

Excess amount of cobalt has negative effects on methanogenesis. Cobalt concentration of 950 mg/l led to 100 % inhibition in anaerobic toxicity bioassays (Bhattacharya et al. 1995), while no inhibitory concentrations on UASB reactor have been reported.

2.4.2.4 Nickel

Nickel has different functions in anaerobic reactions, such as CO-dehydrogenase, acetyl-CoA synthase, methyl-CoM reductase, urease, hydrogenase, and stabilator of DNA and RNA (Fermoso et al. 2008). Nickel supplementation in UASB reactors alongside cobalt and iron at a temperature of 35 ^{o}C has led to better reactor performance. Observations about increased COD removal (Shen et al. 1993) and better sludge growth and sludge retention (Oleszkiewicz et al. 1989) have been made. Increased COD removal has also been noted with supplementation of nickel, cobalt, and molybdenum (Espinosa et al. 1995).

High concentration of nickel has had toxic effect on UASB reactor performance. Depending on the conditions, nickel concentration of 81 mg/l and 440 mg/l led to 50 % inhibition of VFA degradation (Lin and Chen 1999). Concentration of 118 mg/l has led to 50 % inhibition of SMA (Fang and Hui 1994) and concentration of 78 mg/l has led to 50 % inhibition of SMA (Lin and Chen 1997).

2.4.2.5 Selenium, molybdenum, and tungsten

Selenium, molybdenum, and tungsten have different roles in anaerobic reactions. Selenium acts as a hydrogenase and formate dehydrogenase, molybdenum acts as a co-factor for dehydrogenases for propionate oxidizing bacteria, and tungsten acts as a co-enzyme in anaerobic biochemical reactions helping to maintain anaerobic processes (Fermoso et al. 2008). Also, methanogens growing on H_2 and CO_2 require tungsten (Jiang et al. 2012). Long-term absence of selenium, molybdenum, and tungsten have been found to decrease activity of propionate degrading community in alcohol distillery wastewater fed UASB reactor (Worm et al. 2009).

2.4.2.6 Zinc

Zinc is a co-factor for enzymes in anaerobic reactions, which makes it an essential trace element for micro-organisms, especially in methanogenesis (Fermoso et al. 2008). Osu-

na et al. (2002) studied metal deprivation in VFA fed UASB reactors and found that when adding solely zinc to the medium, the SMA with acetate as the substrate of metal deprived sludge increased by 36 %.

High concentration of zinc has had negative effects on reactor performance, mostly in methanogenic activity. 50 % inhibition in methanogenic activity in UASB reactor's granular sludge have been found at zinc concentration of 690 mg/l (Hydraulic retention time (HRT) 1 day) and 270 mg/l (HRT 2 days) wine wastewater as substrate (Lin and Chen 1999), and 96 mg/l starch synthetic wastewater as substrate (Fang et al. 1997).

2.4.2.7 Manganese and magnesium

Manganese acts a co-factor for enzymes and is an essential trace element for micro-organisms in anaerobic reactions. It stabilizes methyltransferase in methane-producing bacteria and is a stimulator for the growth of certain methanogens (Fermoso et al. 2008). According to Osuna et al. (2002), supplementation of sole manganese improves acetate- and propionate degradation.

Magnesium is required for stimulating growth for several methanogens. It has been studied that for some species, magnesium shortens the generation time, although, it has-n't been proven that it would increase the final cell yield (Singh et al. 1998). Chen et al. (2008) in their review states that in *Methanosarcina* -dominated UASB reactor optimal concentration of magnesium is reported to be around 720 mg/l.

2.4.2.8 Sodium

Sodium requirement varies widely amongst the different methanogens (Singh et al. 1998 review). Most methanogens are reported to possess an inwardly directed gradient of sodium ions (Na^+) – a requirement of sodium for both growth and CH_4 production. Sodium's bioenergetic role for methanogens is quite well known. Amino acid transport, growth, internal pH regulation and even the methanogenesis are all dependent for sodi-um. (Jarrell et al. 1988).

Sodium concentration of 100–350 mg/l has been found to have positive effects on UASB reactor performance (Singh et al. 1998 review). Concentrations from 3500 mg/l to 8000 mg/l of sodium have been found to be strongly inhibitory for methanogens of mesophilic atmosphere (Chen et al. 2008) but there are no recent studies considering the positive effects of sodium in UASB reactor performance.

2.4.2.9 Potassium and chromium

Micro-organisms require potassium for normal growth (Pelczar et al. 1993). Potassium concentration of 400 mg/l or less has been found to enhance UASB reactor performance in mesophilic ranges. Higher concentrations have had inhibitory effects, which was more pronounced in the thermophilic temperature range (Chen et al. 2007).

Chromium is considered as a heavy metal and experiments indicate that so far its effect on UASB reactor performance has been negative (Alkan et al. 1995; Lin and Chen 1998; Altas 2008). According to Alkan et al. (1995), soluble trivalent chromium with a concentration of 500 mg/l in a shock injection led to process failure. A shock injection of 400 mg/l did not result in a failure of the process, although a reduction in the performance of the reactor was observed.

3 MATERIALS AND METHODS

The objective of this study was to find out the effects of added trace elements on the UASB reactor performance. The experiment consisted of two periods: Adaptation period and study period. During the adaptation period one UASB reactor was fed with brewery wastewater. In the study period, five UASB reactors were fed with same wastewater, but with added trace elements containing nutrient solutions.

3.1 Wastewater, additives, and inoculum

The feed used in the experiment was brewery wastewater from Sinebrykoff (Espoo, Finland). Wastewater was received on a weekly basis every Tuesday and was stored in canisters at 4 ^{o}C during the experiment. Wastewater pH was 6.4-6.7 and soluble chemical oxygen demand (SCOD) 1.70-3.70 g/l. Totally 36 wastewater batches were used in the experiment.

The feed for the reactors was prepared 1-4 times per week depending on the flow velocity and the sizes of influent and effluent containers. During the study period, the feed of four UASB reactors was supplied to contain different trace elements containing nutrient solutions, which consisted of four different nutrient solutions: Solution BDP-881, solution A, solution B, and solution C.

Solution BDP-881 consisted of Fe, Co, Ni, Se, and Mo. Solution A consisted of BDP-881 and Zn, solution B consisted of BDP-881, Zn, and Mn, and solution C consisted of BDP-881, Zn, Mn, and Ca. The concentrations of each element in the nutrient solutions are shown in table 3.1. The added concentrations were determined according to recommended metal concentrations in anaerobic digestion processes by Speece (1996). Table 3.2 shows compounds of each nutrient solution.

Table 3.1. *Element concentrations in the nutrient solutions*

Element	Concentration (g/l)
Fe	13.860
Co	0.0308
Ni	0.0462
Se	0.00616
Mo	0.0231
Zn	0.0770
Mn	0.0385
Ca	3.080

Table 3.2. *Solution compounds used in the study period*

Solution	Elements
BDP-881	Fe, Co, Ni, Se, Mo
A	Fe, Co, Ni, Se, Mo & Zn
B	Fe, Co, Ni, Se, Mo & Zn, Mn
C	Fe, Co, Ni, Se, Mo & Zn, Mn, Ca

The inoculum was granular sludge, obtained from a full scale UASB reactor treating bioethanol production waste (Jokioinen, Finland). The sludge was stored for about two weeks in sealed containers at 4 ^{o}C before added into the reactor in adaptation period. The granular sludge was washed with six liters of tap water to remove non-settling biomass.

3.2 Reactor setup

The experiment consisted of two periods: Adaptation period and study period. During the adaptation period, one reactor was operated and during study period five reactors were operated. Setup for both periods was similar.

During the adaptation period a PVC reactor with volume of 3.3 liters (height 50 cm, diameter 10 cm) was used, while during the study period five 450 mL glass reactors (height 25 cm, diameter 5 cm) were used. In both periods, the feeding tank filled with brewery wastewater was kept in a refridgerator at 4 ^{o}C and was connected to an aluminum bag filled with pure nitrogen. A hydraulic pump (MasterFlex easy-load X, Model 77800-60) was used to feed the reactor.

The reactor in adaptation period was wrapped with a heated coil with flowing water inside and the reactor temperature was kept at 35 ^{o}C. Effluent was collected in a plastic canister. During the study period the reactors were kept in the same heat pool filled with water at 35 ^{o}C, and effluent containers were glass bottles. During both periods the pro-

duced biogas was collected into aluminum bags above the reactors. Also, in adaptation- and study period, MasterFlex –rubber hoses were used to connect the feeding tank and aluminum bags to the reactors. Figures 3.1 and 3.2 illustrate the adaptation period setup and figures 3.3 and 3.4 illustrate the study period setup.

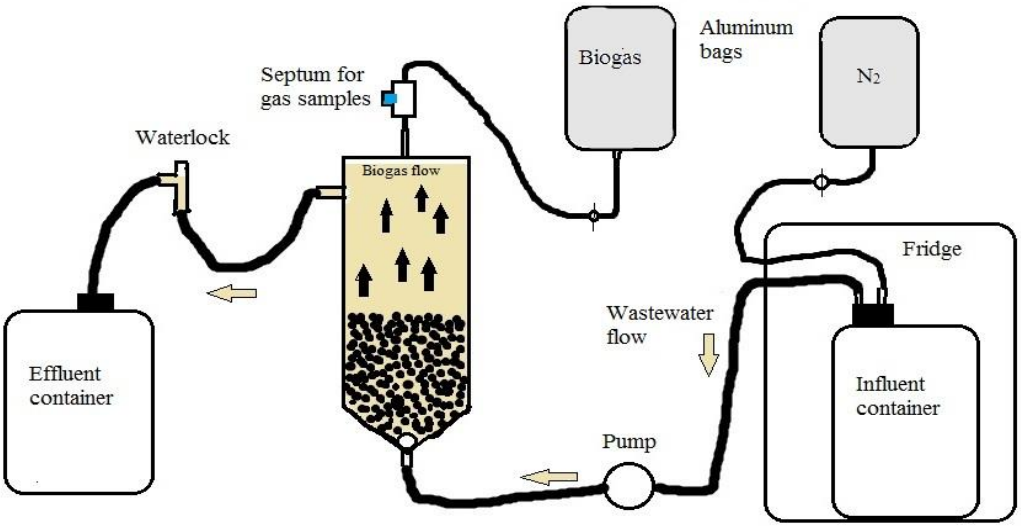


Figure 3.1. *Adaptation period setup in the treatment of brewery wastewater*

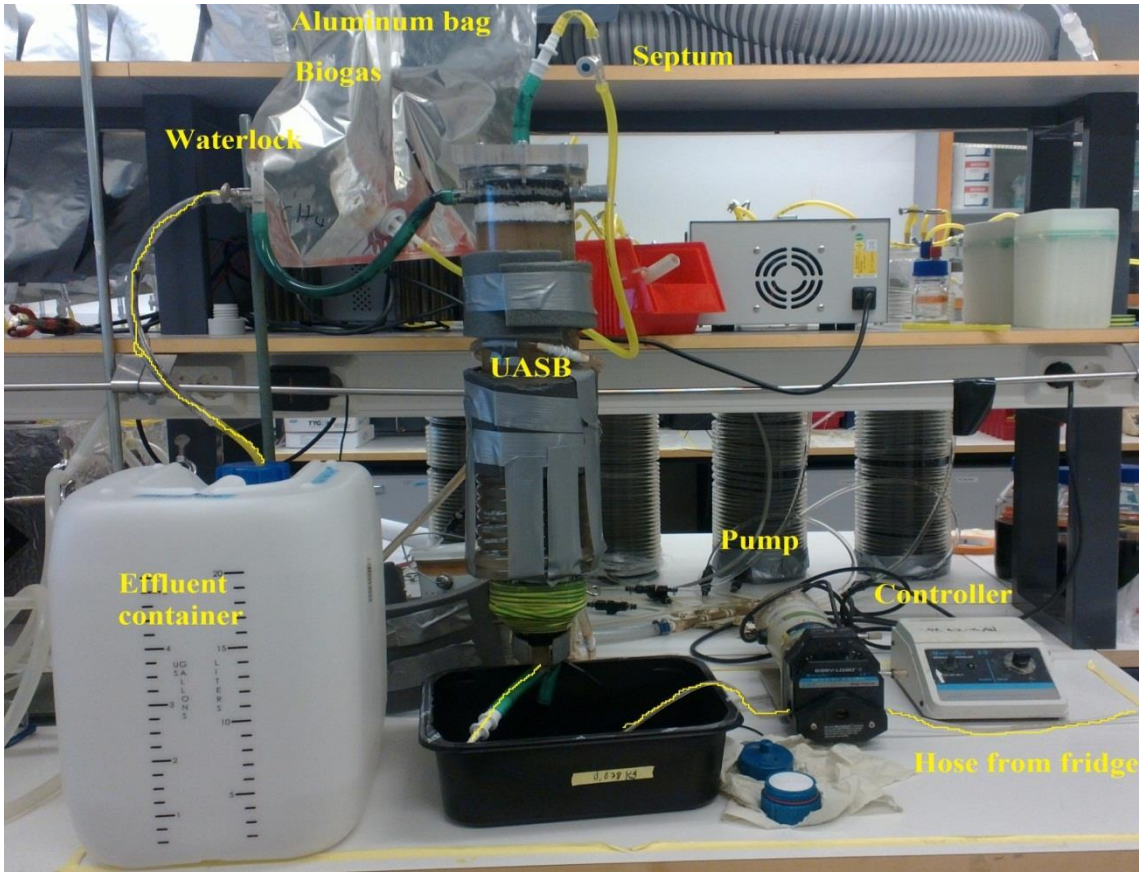


Figure 3.2. *Adaptation period setup*

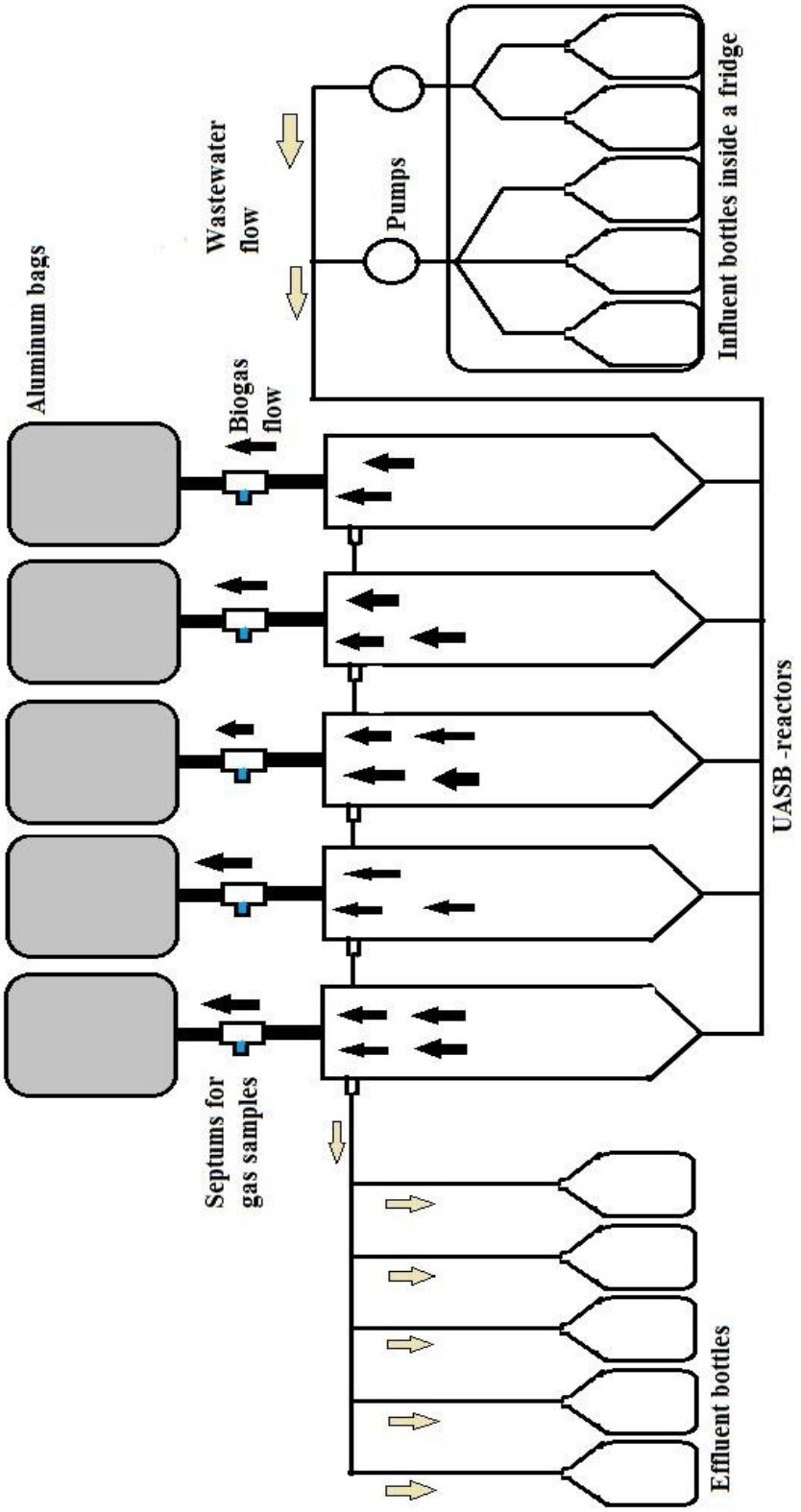


Figure 3.3. *Setup in the study period for studying the effects of additives on the treatment of brewery wastewater in UASB reactors. Water locks and heat pool are not depicted in the figure.*

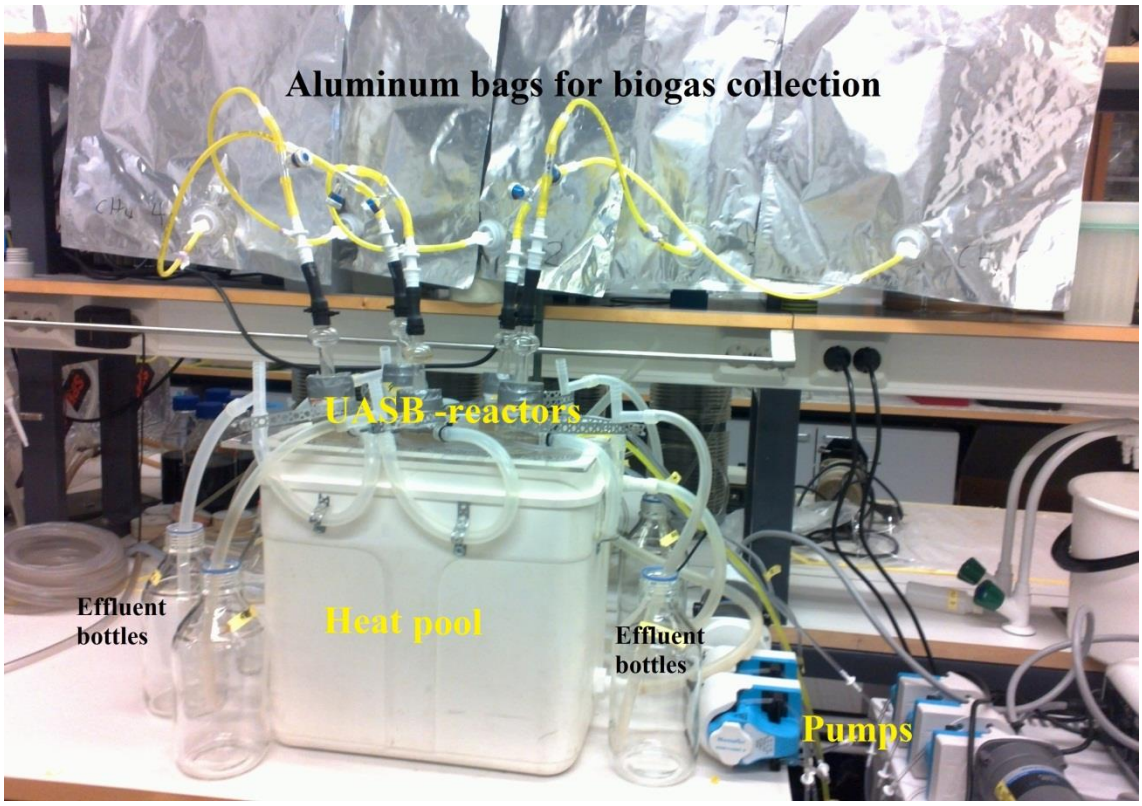


Figure 3.4. *Study period setup in the treatment of brewery wastewater*

3.3 Operation strategy

During the adaptation period, one UASB reactor was operated and fed with brewery wastewater for 27 days. The purpose of this period was to adapt the microbial population to the substrate used in the experiment later in the study period.

Before adding the granular sludge, the reactor was flushed with nitrogen. Total mass (wet weight) of added granular sludge was 1290 g (58 g of volatile solids (VS)). After the sludge had been added the reactor was filled with the feed and then the reactor was activated by starting the pumps. OLR and HRT were elevated and decreased, respectively, stepwise during the adaptation period. OLR was 2.5 g SCOD/l/d in the beginning and 4.3 g SCOD/l/d in the end (day 27). HRT was 28.4 h in the beginning and 15.9 h in the end of the adaptation period. After the adaptation period, the granular sludge was kept at 4 ^{o}C in a sealed container for five days, before used in the study period.

At the beginning of the study period, the granular sludge from the adaptation period reactor was divided into five 0.450 L UASB reactors. Again, before adding the sludge and the feed into the reactors, the reactors were flushed with nitrogen. Masses of granular sludge added into each UASB reactor in beginning of the study period were 200 – 214 g (wet weight).

All the five reactors were fed with the same brewery wastewater as used in the adaptation period. One reactor was kept as a control reactor and was fed with the wastewater, while the other four reactors were fed with wastewater containing different nutrient solutions additions. The addition strategy is shown in table 3.5.

Table 3.3. *The strategy for nutrient solution addition in UASB reactor in the study period*

Day	R1	R2	R3	R4	R5
1-8	-	-	-	-	-
9-17	-	BDP-881*	BDP-881	BDP-881	BDP-881
18-79	-	BDP-881	A*	B*	C*
80-83	BDP-881	BDP-881	A	B	C

** BDP-881 contained Fe, Co, Ni, Mo, and Se; sol. A contained sol. BDP-881 and Zn; sol. B contained sol. BDP-881, Zn, and Mn; sol. C contained sol. BDP-881, Zn, Mn, and Ca.*

The target volume of the added nutrient solution in the feeding tanks of reactors R2, R3, R4, and R5, was calculated with formulas 3.1, 3.2, and 3.3 in chapter 3.4.

During the study period, OLR and HRT were meant to be kept at the same rate between the reactors, and were elevated and decreased, respectively, stepwise during the study. This was carried on, until the OLR was so high that the CH_4 production would decrease, and to find out if the nutrient solutions had any effects to the reactor performance. Table 3.4 shows the OLR and HRT values for each reactor in the beginning and in the end of the study period.

Table 3.4. *OLR and HRT values in the beginning and in the end of study period*

	OLR (g SCOD/l/d)		HRT (h)	
Reactor	**Day 1**	**Day 83**	**Day 1**	**Day 83**
R1	4.5	17.3	19.9	3.1
R2	4.5	17.3	19.9	3.1
R3	3.8	18.5	23.2	2.9
R4	4.7	17.3	19.1	3.1
R5	5.0	17.6	17.7	3.1

3.4 Analyses and calculations

Total solids (TS) and VS were analyzed according to standard methods (APHA, 1998). pH was measured with WTW's pH-3210 pH-meter, immediately from the effluent samples. COD was analyzed according to Finnish Standard Methods (SFS 5504, Finnish Standard Association, 1988). For SCOD, the samples were filtrated with Whatman Glass Microfibre GF/A filters.

VFAs were determined by using a gas chromatograph (Shimadzu GC-2010, FID1 detector, Capillary GC Column ZB-wax plus 30 m x 0.25 mm x 0.25 µm). Helium was used as a carrier gas and operation conditions were: Detector 250 °C, injector 250 °C, column 40 °C, injection volume 1 µl, total column flow 82.9 ml/min. Program condition were: 2 min hold at 40 °C, rate 20 °C/min till 160 °C, rate 40 °C/min till 220 °C. VFA samples were filtrated with Chromafil Xtra Pet-45/25 0.45 µm filters.

Gas composition (CH_4 and CO_2) was measured by using a gas chromatograph (Shimadzu GC-2014, Agilent Porapak n80-100 mesh 1.8 m x 2 mm column). Helium was used as a carrier gas and operation conditions were: Injector 80 °C, detector 80 °C, column 40 °C, column flow 25 mL/min. Biogas volume was measured by water displacement method. Biogas results were converted to standard temperature and pressure conditions (T = 296 K, p = 1.013 bar).

For both adaptation and study period, the same analyses were performed for each UASB reactor. Analyses and weekly schedules are listed in the table 3.6.

Table 3.6. *List of analyses and weekly schedules during the experiment*

Analysis	Mo	Tu	We	Th	Fr
Effluent volume	X	X	X	X	X
Gas volume	X	X	X	X	X
CH_4 %	X		X		X
Effluent COD	X		X		X
Effluent VFA	X		X		X
Effluent pH	X		X		X

Besides the analyses in table 3.6, TS and VS were determined for granular sludge before and after the adaptation period, and for the granular sludge and the supernatant of each reactor after the study period. Influent COD and VFA samples were also collected and analyzed from every new feeding tank during the whole experiment.

Volume of added BDP-881 solution was determined according to recommended cobalt concentration (0.003 mg/g COD; 0.02 mg/l excess) from the previous literature (Speece 1996). Target cobalt concentration was 30 mg/l, volume of feeding tanks was 5 l, cobalt concentration in BDP-881 solution was 200 mg/kg and density of BDP-881 was 1.1 g/l. Mass of needed cobalt addition was calculated according to formula 3.1

$$m(Co)need = \frac{c(Co) * V(feed)}{1000\ L} \quad (1),$$

where $m(Co)_{need}$ is mass of needed cobalt, $c(Co)$ is target cobalt concentration, and $V(feed)$ is the volume of the feeding tank. Mass of dosed cobalt into the feeding tank was calculated according to formula 3.2

$$m(Co)dose = \frac{m(Co)need}{c(Co)BDP} * 1000 \quad (2),$$

where $m(Co)_{dose}$ is mass of dosed cobalt into the feeding tanks and $c(Co)_{BDP}$ is cobalt concentration in BDP-881 solution. Volume of added BDP-881 solution into the feeding tanks was calculated according to formula 3.3

$$V(additive) = \frac{m(Co)dose}{\varrho(BDP)} \quad (3),$$

where $V(additive)$ is volume of added BDP-881 solution into the feeding tank and $\varrho(BDP)$ is density of BDP-881 solution.

3.4.1 Metal analyses

Metal concentration analyses were performed for effluent samples. The samples were taken on days 35, 46, 54, 66, 76 and 82. Granular sludge samples were taken on days 1 and 83. Analyses were performed by Kemira in Espoo, Finland.

5 g of sample was dissolved with nicric acid (HNO_3) (2.5 mL) and hydrogen peroxide (H_2O_2) (2 ml). After dissolving samples were cooled down to room temperature and stabilized with HCl (0.5 mL) and diluted to 50 ml. Samples were filtrated with 0.45 µm filters. Iron and calcium were analyzed with ICP-OES, and cobalt, nickel, selenium, manganese, and zinc were analyzed with ICP-MS.

ICP-OES analysis was performed with PerkinElmer Optima 5300 DV. Wave lengths for iron and calcium were 238 nm and 317 nm, respectively. Iron and calcium analyses were performed semi-quantitatively and inner standard was 2 mg/l of Sc. Multiple element calibration standards were 0-10 mg/l of iron and calcium (5 v/v % HNO_3 matrix).

ICP-MS analysis was performed with Agilent 7700x. Isotopes were ^{59}Co, ^{60}Ni, ^{78}Se, ^{66}Zn and ^{55}Mn and inner standards were ^{45}Sc, ^{89}Y, and ^{115}In (1000 µg/l). Multiple element calibration standards were 0-100 µg/l of cobalt, nickel, selenium, and manganese, and 0-500 µg/l of zinc (5 v/v % HNO_3 and 1 v/v % hydrochloric acid).

4 RESULTS

4.1 Adaptation period

During the adaptation period, HRT was decreased stepwise from 28.4 h to 15.9 h. OLR was 2.5 g SCOD/l/d in the beginning and 4.3 g SCOD/l/d in the end, but it was higher during days 15-21 (5.0-5.1 SCOD/l/d). During the adaptation period, influent SCOD varied from 2.8 to 3.6 g/l and influent VFA from 0.9 to 1.4 g/l. Effluent SCOD was 0.55 g/l in the beginning, and gradually decreased to 0.25 g/l in the end, and effluent VFA varied between 0.052-0.166 g/l. CH_4 content varied from 55 to 81 %, and CH_4 production varied between 2.1-6.2 l/d and the highest values were measured on day 15. pH varied between 7.1-7.9.

4.2 Study period

Result in the study period are presented in the following section: HRT, OLR, gas composition and CH_4 production in figure 4.1, concentrations of influent and effluent COD, COD removal, and CH_4 production per removed SCOD g per day in figure 4.2., and pH values and VFA concentration are presented in figure 4.3.

During days 1-8, reactors were fed with only wastewater. HRT was gradually decreased from 18-23 h to 15 h. OLR was between 4-5 g SCOD/l/d. CH_4 production varied from 0.6 to 0.7 L/d and there were no major differences between the reactors. VFA, COD removal, and pH were also at the same level between the reactors. During these first days effluent VFA was below detection limit, COD removal between 92-94 %, and pH 8.3 – 8.5 in every reactor.

During days 9-17, BDP-881 nutrient solution was added to reactors R2-5. HRT was kept between 14-16 h in each reactor. OLR elevated over 5.7 g SCOD/l/d in every reactor on day 10, but decreased after that and during days 12-14 the values were between 2.7-3.5 g SCOD/l/d in every reactor. CH_4 production was around 6 l/d in every reactor. pH, VFA, and COD removal stayed at the same level than during days 1-8.

From day 18 to 83 solutions BDP-881, A, B, and C were added to the feed of R2-5 according to table 3.2. During days 18-38, HRT was decreased from 15 h to 10 h in every reactor. There were no major differences between reactor performances until on day 20. CH_4 production was 0.4-0.7 l/d in each reactor, except in R3. CH_4 production dropped to 0.25 l/d and on day 21 it was near 0 l/d in R3. During days 22-31 R3 was fed with 1:1

diluted wastewater and OLR was 2.0-2.4 g SCOD/l/d, whereas in other reactors OLR was elevated from 4.4 (day 22) to 5.7 (day 31) g SCOD/l/d. During days 21-24 pH in R3 was under 6, whereas in other reactors pH was over 8. During days 25-33 pH in R3 fluctuated between 7.4 and 7.9, but on day 35 pH values reached over 8.3; same level than in other reactors. During days 18-38, effluent VFA concentration in R1, R2, R4, and R5 was less than 0.4 g/l, while in R3 effluent VFA varied between 0.13 and 0.48 g/l. During days 18 – 38 COD removal was 89-96 % in every reactor, expect in R3. On day 31, COD removal was 72 % and on day 35, it was 57 %. On day 38, COD removal in R3 was 87 %, reaching the same level than the other reactors.

During days 39-59, HRT was decreased from 10 h to 7 h or below, OLR was elevated from under 5.0 g SCOD/l/d to over 9.0 g SCOD/l/d, and CH_4 production increased from under 0.5 l/d to over 1.3 l/d. pH decreased gradually slightly below 8 and COD removal was 83-96 %. Only notable differences were in VFA concentrations. VFA was less than 0.1 g/l in reactors R1, R2, R4, and R5 during days 39-50, and during days 51-59 VFA elevated gradually from below detection limit to slightly under 0.2 g/l. In R3 there were some VFAs (0.10-0.25 g/l) during days 45-49, but values decreased to same level with other reactors on day 59.

During days 60-83, HRT was gradually decreased in every reactor to 3 h (day 83). OLR in every reactor elevated from 9.0 (day 60) to 17.6 (day 83) g SCOD/l/d, but there were some fluctuation. For example, on day 74, the highest value was measured (19.1 g SCOD/l/d). CH_4 production elevated gradually from 1.3 l/d (day 60) to 2.4-2.6 l/d (day 83) in reactors R2-5. CH_4 production in R1 decreased and it was 0.7-0.9 l/d during days 64-68 and 0.5 l/d on day 74.

During days 75-77 R1 didn't produce CH_4 at all. During days 80-83, R1 was fed with solution BDP-881. The addition recovered the reactor, and it started to produce CH_4 again (1.7 l/d on days 82 and 83). pH in reactors R2-5 was 7.4-8.2 during days 60-83. In R1, pH dropped to 7.0 on day 64 and kept decreasing down to 6.8 (day 75). BDP-881 addition caused pH to elevate slightly over 7 in the end of the experiment. During days 60-83 COD removal in reactors R2-5 varied between 81 and 94 % and there weren't notable differences between these reactors. On day 61, COD removal in R1 was 76 % and the removal rate kept decreasing: 48 % on day 66, 36 % on day 68, 19 % on day 75, and 7 % on day 77. BDP-881 addition increased the COD removal and it elevated to 43 % on day 81 and to 59 % on day 83. On day 60, there were some differences in VFA concentrations between the reactors: 0.37 g/l in R1, 0.21 g/l in R2, 0.13 g/l in R3, 0.25 g/l in R4, and 0.12 g/l in R5. During days 61-74 VFA concentration varied between 0.09 and 0.21 g/l in reactors R2-5. In R1, VFA kept increasing, and from day 64 to day 74 VFA elevated from 0.72 g/l to 0.89 g/l. During days 75-83 VFA concentration in R2-5 was below 0.20 g/l. In R1, VFA decreased from 1.05 g/l (day 77) to 0.55 g/l (day 83).

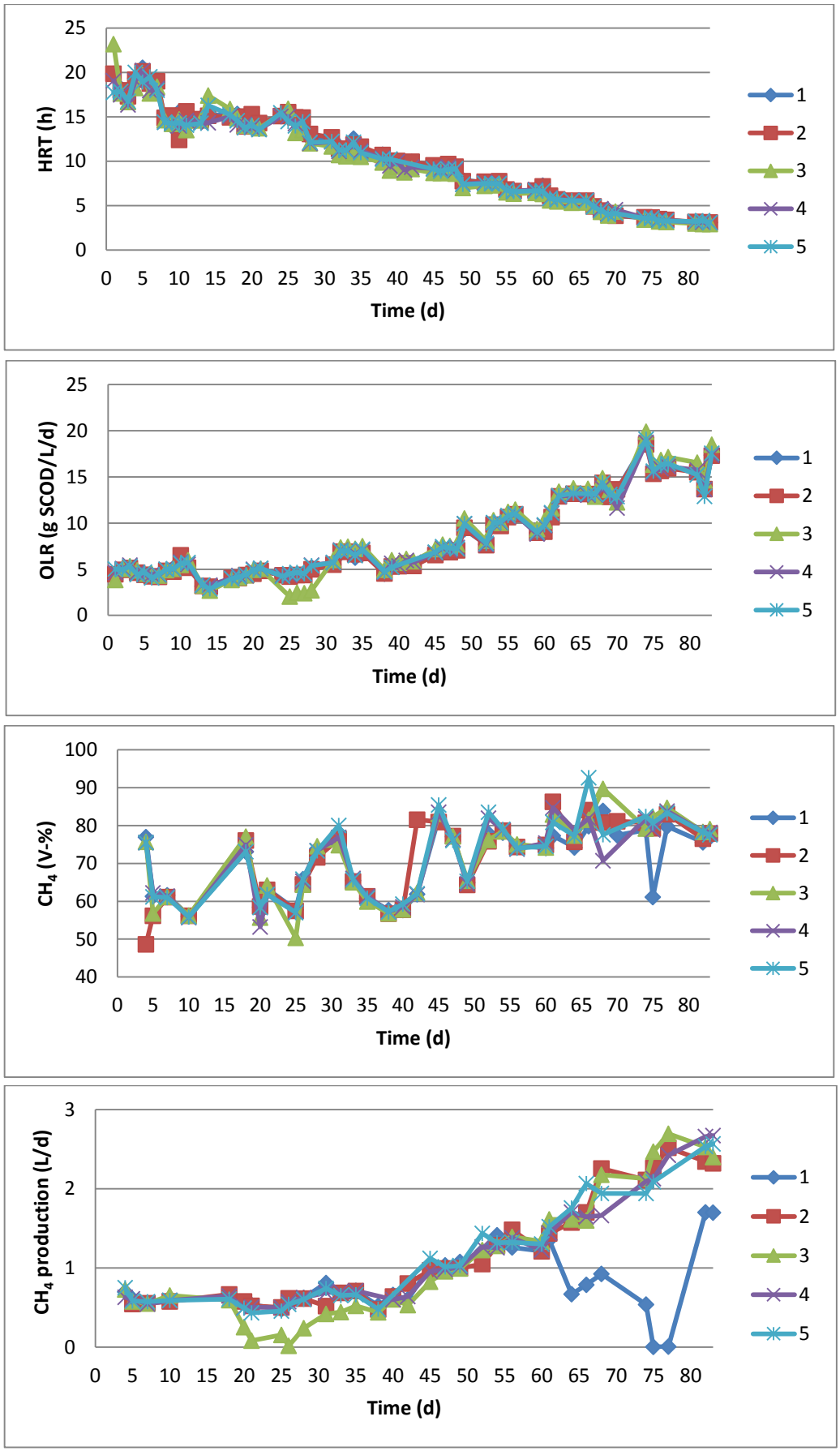


Figure 4.1. *HRT, OLR, gas composition, and daily CH_4 production in five UASB reactors during study period.*

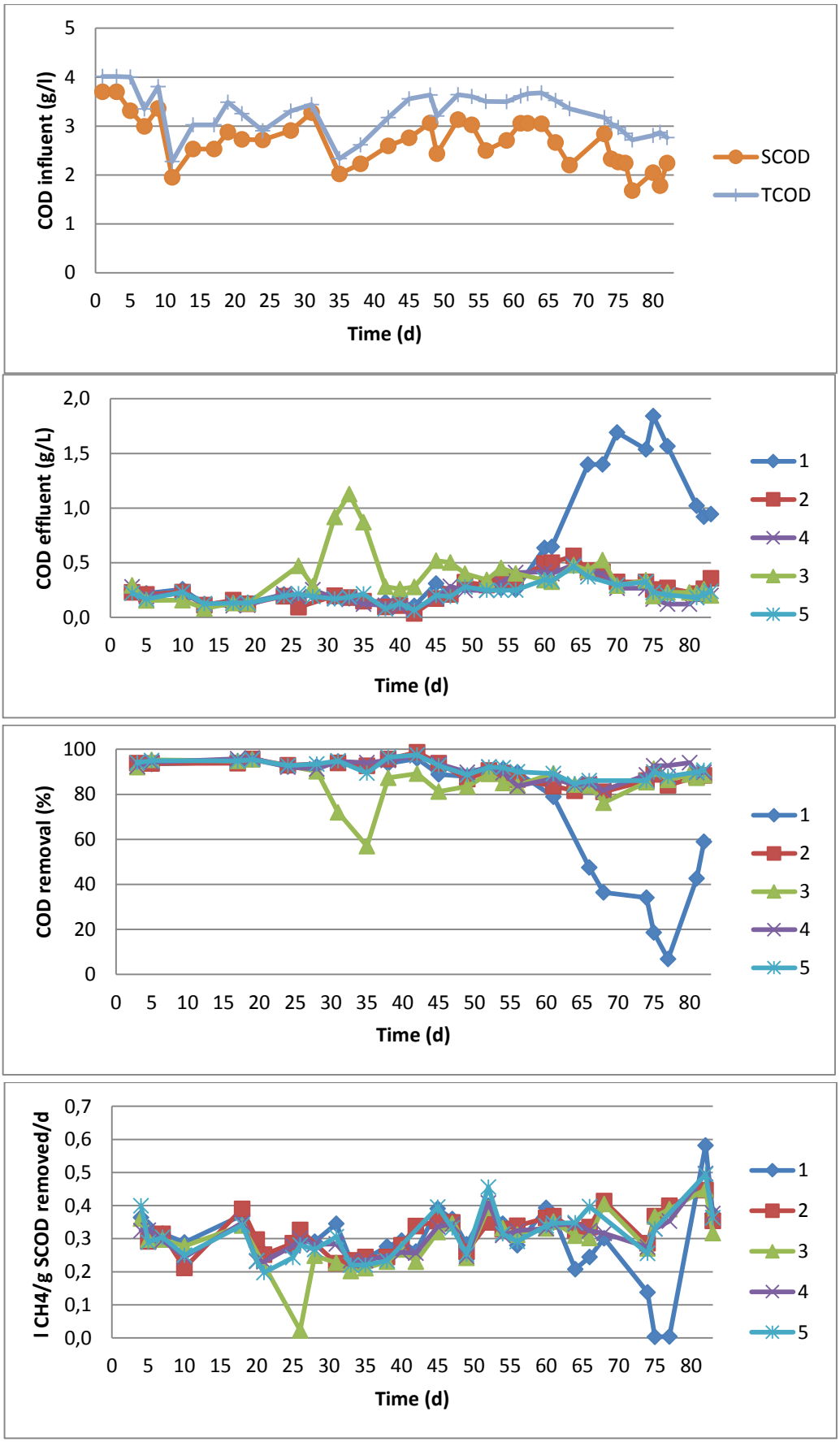
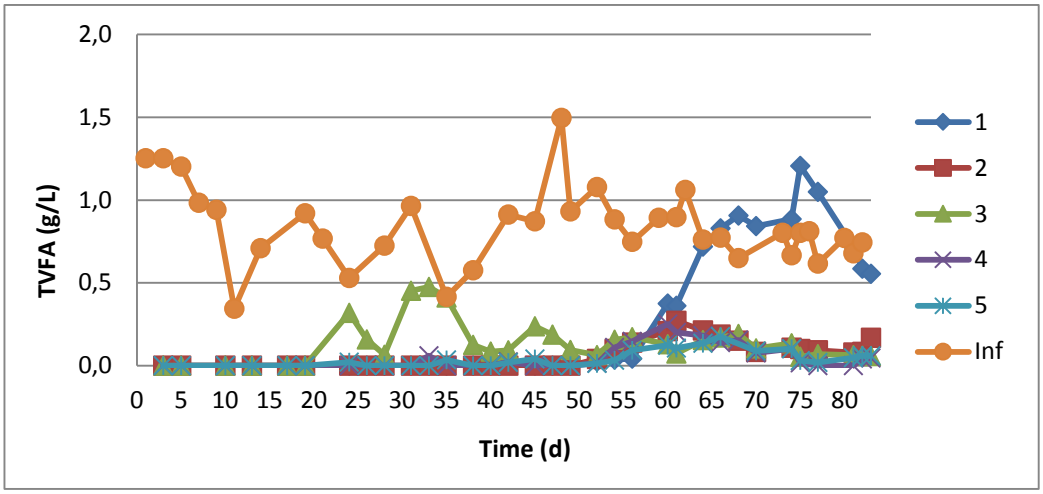


Figure 4.2. *Influent and effluent COD concentration, COD removal, and daily CH_4 production per removed SCOD g in five UASB reactors during study period.*



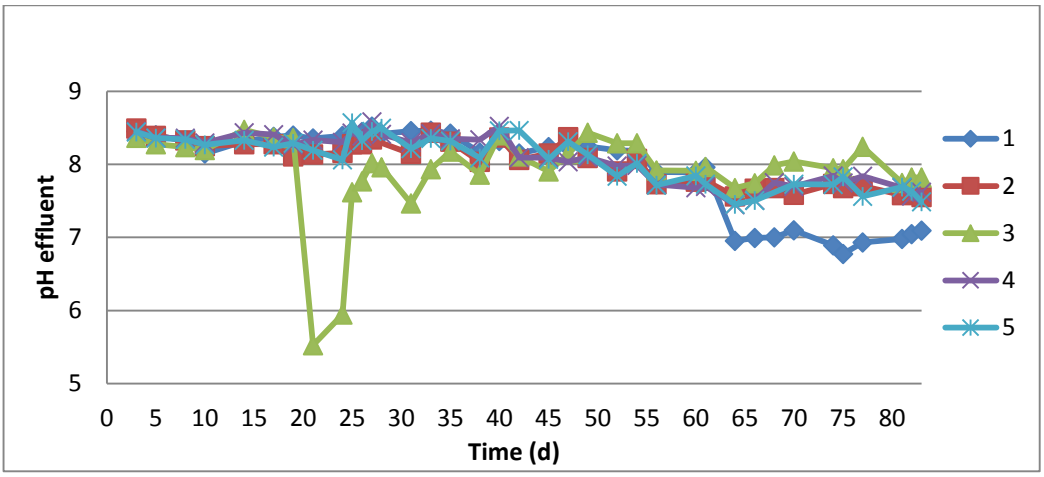


Figure 4.3 *pH and VFA concentrations in five UASB reactors during study period.*

4.3 Granular sludge characteristics and trace elements

After the study period, the granular sludge inside the reactors was studied. TS and VS analysis results are listed in table 4.1 and sludge masses are listed in table 4.2.

Table 4.1. *TS and VS of the granular sludge before and after the study period*

Day	Sample	Reactor	TS-%	VS-%	VS/TS-%
1	Granula before study period	Adaptation	5.1	4.5	87.0
83	Granula after study period				
		R1	4.7	4.2	89.6
		R2	4.6	3.9	85.0
		R3	4.1	3.5	85.3
		R4	6.2	5.3	85.4
		R5	6.0	5.2	86.2

Table 4.2. *Masses of granular sludge on day 1 and day 83, change of mass of granular sludge in the reactor.*

Reactor	Sludge mass day 1 (g)			Sludge mass day 83 (g)			Change in sludge mass (g)		
	Wet	**TS**	**VS**	**Wet**	**TS**	**VS**	**Wet**	**TS**	**VS**
R1	214	10.9	9.6	200	9.4	8.4	-14	-1.5	-1.2
R2	201	10.3	9.0	196	9.0	7.6	-5	-1.3	-1.4
R3	200	10.2	9.0	199	8.2	7.0	-1	-2.0	-2.0
R4	203	10.5	9.1	169	10.5	9.0	-34	0	-0.1
R5	200	10.3	9.0	172	10.3	8.9	-28	0	-0.1

As the table 4.2 shows, the mass of changed granules was the smallest in R3. The biggest change in the mass was in R4. VS/TS ratio varied only a little and didn't seem to be affected, whether trace elements were added or not.

4.4 Metal analyses

Effluent trace element concentrations are presented in Appendix 1. Trace element concentrations in effluent samples were significantly lower in R1 than in R2-5 during the study period. Only on day 82 they were higher, which can be explained by the addition of BDP-881 in R1 after the reactor failure had occurred. On days 35, 46, 54, 66, and 76, iron concentration in R1 was approximately 1 mg/l, but on day 82 the concentration was 29 mg/l. In R2-5 iron concentration was 7-9 mg/l on days 35, 46 and 54, and 9-14 mg/l on days 66, 76, and 82. Cobalt concentration in R1 was below detection limit on days 35, 46, 54, 66, and 76, and 64 µg/l on day 82. Cobalt concentration in R2-5 was 26-32 µg/l during the whole experiment. Nickel concentration in R1 was 2-3 µg/l on days 35, 46, 54, 66, and 76 and 54 µg/l on day 82. In R2-5 nickel concentration was 24-40 µg/l during the experiment.

Trace element loadings didn't fluctuate significantly during the experiment. There was a slight upward trend in every trace element load, but it can be explained with the stepwise elevation of OLR during the experiment. Results for added trace element per SCOD are presented in Appendix 2.

5 DISCUSSION

In this study, trace element addition into the feed of UASB reactor enabled higher OLR, CH_4 production, and COD removal than without trace element addition. Also, addition of trace elements into the feed helped the control reactor to recover and to start the halted CH_4 production again. Furthermore, the results showed that essential trace elements for better reactor performance were most probably in the BDP-881 solution, because supplementation of zinc, manganese, and calcium didn't seem to have any effects on the reactor performance.

The highest OLR values with BDP addition were over 18.5 g SCOD/l/d (day 74) and there were no signs of decreased CH_4 production or reactor failure at that OLR in R2-5. In R1, CH_4 production started decreasing, when OLR was 13-14 g SCOD/l/d on day 64. Espinosa et al. (1995) got similar results in molasses stillage fed UASB reactor. VFA accumulation and decreasing COD removal were notified in the reactor at 17.4 g SCOD/l/d of OLR in UASB reactor in absence of iron, cobalt, nickel, and molybdenum in the feed. Also, Osuna et al. (2003) had found that lack of trace element led to decreased VFA conversion and COD removal in alcohol distillery wastewater fed UASB reactors at 10 g SCOD/l/d of OLR.

Trace element addition into the feed helped R1 to recover. BDP-881 was added in the feed of R1 on day 80, and the reactor recovered in three days and CH_4 production rose to 1.7 l/d from 0 l/d. COD removal increased from 7 % to 43 % and VFA concentration decreased from 1.1 g/l to 0.6 g/l. Earlier studies also indicate that trace element addition in the feed may have recovery effects. For example, Espinosa et al. (1995) added iron (100 mg/l), nickel (15 mg/l), cobalt (10 mg/l) and molybdenum (0.2 mg/l) in the feed of cane molasses stillage fed UASB reactor, and the COD removal increased from 44% to 58% and the biogas production from 10.7 to 14.8 l/d. Level of propionic acid and acetic acid reduced from 5.29 g/l to 0.25 g/l and from 1.10 g/l to 0.16 g/l, respectively. Also, addition of cobalt (295 µg/l) in the feed of methanol fed UASB reactor has been studied to triple the CH_4 production in one day (Fermoso et al. 2008).

The essential trace elements for increased reactor performance were most probably in BDP-881 solution, because between reactors R2-5, there were no major differences during the last days of the experiment. Also, bare BDP-881 solution addition caused R1 to recover. BDP-881 consists of cobalt, iron, nickel, selenium, and molybdenum. Individu-

al effects of cobalt and iron have been studied earlier, but there are also studies considering effects of several trace elements to the UASB reactor performance.

Concentration of cobalt in the effluent of R2-5 was 25-31 µg/l from the first BDP-881 addition to the end of the study period. Such cobalt concentration enabled reactors R2-5 to be operated at over 65 % higher OLR compared to the reactor without cobalt addition. Earlier it has been studied that long-term addition of cobalt (19 µg/l in effluent) increased substrate utilization rate from 55 % to 100 % in methanol fed UASB reactor (Zandvoort et al. 2002). Also, single cobalt addition (1.8 µg/l) into the feed of methanol fed UASB reactor increased CH_4 production by a factor of four in 20 days (Zandvoort et al. 2004). According to Florencio et al. (1993), cobalt supplementation into the medium provided three times more CH_4 production compared to non-supplemented, methanol fed UASB reactor.

Concentration of iron in the effluent of R2-5 was 8-13 mg/l from the first BDP addition to the end of the study period. Vlyssides et al. (2008) studied two synthetic milk wastewater fed UASB reactors with and without iron supplementation. Iron load was increased stepwise from 0.014 to 0.100 g Fe^{2+}/l/d (effluent concentration not determined). Iron-supplemented reactor's COD removal was nearly 100 % during the whole experiment, whereas the non-supplemented reactor's COD removal decreased steadily to 75 % until the end of the experiment. In batch experiments studying inhibitory effects of iron, it was noticed that iron concentration up to 450 mg/l in ethanol fed UASB reactor provided 40 % higher substrate utilization, compared to non-fed reactor (Gonzales-Silva et al. 2009).

As mentioned, cobalt and iron may individually improve UASB reactor's performance, but BDP-881 also contained nickel, selenium, and molybdenum (24-40 µg/l, 1-3 µg/l, and 4-12 µg/l, respectively) in R2-5 during the study period. Several earlier experiments considering the addition of multiple trace elements in UASB reactors have included at least cobalt or/and iron, but recently it has been reported other vital trace elements for UASB reactors as well. For example, a long-term absence (over 250 days) of molybdenum, tungsten, and selenium from the feed of alcohol distillery wastewater fed UASB reactor have been studied to decrease CH_4 production (Worm et al. 2009).

Positive effects of added trace elements in the present study are likely to be correlated to the essential function of metals as co-factors in enzyme system active in the anaerobic digestion process. Cobalt is a co-factor of methyltransferases (Roth et al. 1996) and carbon monoxide dehydrogenase (CODH) (Zandvoort et al. 2006). CODH is a key enzyme for the production and consumption of acetate (Hattori et al. 2005). It is also present in both acetogens and methanogens (Karlsson et al. 2012). Iron and nickel are also essential co-factors of CODH, but essential for the activity of several hydrogenases as well, which makes them essential for fermentative micro-organisms (Vignais et al. 2007).

Nickel is also a component of co-factor F_{430}, which is required for the activity of methyl reductase complex that catalyzes the final step of the CH_4 formation pathway (Thauer et al. 2008). Both selenium and molybdenum are formate dehydrogenases. Selenium is also a hydrogenase and glycin reductase and molybdenum is a nitrate reductase and nitrogenase (Fermoso et al. 2008).

Trace element concentrations in R1 effluent were much smaller, than it has earlier been recommended. Before the BDP-881 addition into R1 on day 80, iron, cobalt, and nickel concentrations in the effluent were below 1 mg/l, 1 µg/l, and 3 µg/l, while the recommendations were 10 mg/l, 20 µg/l, and 20 µg/l, respectively (Speece 1996). Iron, cobalt, and nickel concentrations in the effluent of R2-5 were 8-13 mg/l, 25-31 µg/l, and 24-40 µg/l, respectively. Concentrations of molybdenum and selenium in every reactor were under 11 µg/l and 2 µg/l, whereas the recommendations were 50 µg/l and 80 µg/l, respectively (Speece 1996). This may indicate, that molybdenum and selenium addition didn't affect to the improved reactor performance of R2-5.

High OLR caused a failure in R1. VFA accumulation and decreased COD removal indicate that the anaerobic digestion process was limited. Trace element addition clearly enabled higher OLR in R2-5 compared to R1, but between R2-5 there were no significant differences, even though OLR was elevated. Still, it is possible that even higher OLR could have caused differences between reactor performances of R2-5.

Wastewater used in this experiment allowed 13-14 g SCOD/l/d of OLR, until the reactor performance got worse. In trace element fed reactors, OLR peaked at 18.5 g SCOD/l/d without any signs of failure. With similar concentrations of iron, cobalt, and nickel used in this experiment, it could be possible to elevate OLR and improve the reactor performance in full-scale UASB reactors as well.

6 CONCLUSION

According to the results from this experiment, the following conclusions may be drawn:

- Trace element addition into the feed of UASB reactors allowed higher OLR compared to the control reactor. OLR was 18.5 g SCOD/l/d at its highest, which is 37 % higher than the OLR in control reactor before CH_4 production started to decrease. However, maximum OLR without decreased CH_4 production in trace element fed reactors was not determined.
- BDP-881 solution addition into the feed of control UASB reactor helped it to recover in couple of days after a reactor failure. CH_4 production and COD removal rose from 0 l/d to 1.7 l/d and from 7 % to 43 %, respectively. Effluent VFA concentration decreased from 1.1 g/l to 0.6 g/l.
- Elements providing higher OLR in trace element fed reactors were probably in BDP-881 solution, because there were no significant differences in reactor performances between them. Positive effects of added cobalt, nickel, and iron have been studied earlier in similar types of experiments, but with different wastewater. Also, recommended concentrations from literature for cobalt, nickel, and iron were similar compared to the concentrations measured in this experiment. Measured molybdenum and selenium concentrations were significantly lower than recommended in literature, which refers to that essential added elements for better reactor performance may have been cobalt, nickel, and iron.
- Effects of other added elements (zinc, manganese, and calcium) remained unknown in this experiment. However, it is possible that there could have been differences in reactor performances of trace element fed reactors, if OLR would have been elevated even more.

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APPENDIX 1: METAL CONCENTRATIONS OF EFFLUENT SAMPLES

Day 35	mg/l		µg/l					
	Fe	Ca	Co	Ni	Se	Mo	Zn	Mn
R1	1	20	<1	3	<1	2	176	21
R2	7	20	26	28	<1	6	<100	74
R3	8	19	30	34	<1	4	<100	77
R4	9	21	26	26	<1	5	<100	141
R5	7	22	27	28	<1	5	<100	122

Day 46	mg/l		µg/l					
	Fe	Ca	Co	Ni	Se	Mo	Zn	Mn
R1	1	20	<1	3	<1	3	<100	16
R2	8	20	26	25	<1	8	<100	72
R3	8	20	29	32	1	10	<100	80
R4	8	20	26	24	<1	8	<100	137
R5	7	23	25	24	<1	7	<100	120

Day 54	mg/l		µg/l					
	Fe	Ca	Co	Ni	Se	Mo	Zn	Mn
R1	<1	21	<1	2	<1	2	293	13
R2	8	21	29	29	<1	9	257	64
R3	8	20	28	32	<1	9	307	76
R4	7	20	26	34	<1	6	312	120
R5	9	25	26	34	<1	6	237	125

Day 66	mg/l		µg/l					
	Fe	Ca	Co	Ni	Se	Mo	Zn	Mn
R1	<1	20	<1	3	<1	2	376	15
R2	13	20	28	34	2	12	206	64
R3	11	19	30	40	2	11	276	76
R4	10	19	29	33	2	11	343	117
R5	11	23	29	32	1	10	226	117

Day 76	mg/l		µg/l					
	Fe	**Ca**	**Co**	**Ni**	**Se**	**Mo**	**Zn**	**Mn**
R1	1	20	<1	2	<1	2	<100	16
R2	12	20	28	32	<1	6	<100	62
R3	10	20	28	32	<1	6	<100	74
R4	13	20	31	37	3	9	220	132
R5	9	22	26	33	<1	5	<100	111

Day 82	mg/l		µg/l					
	Fe	**Ca**	**Co**	**Ni**	**Se**	**Mo**	**Zn**	**Mn**
R1	29	19	64	54	3	10	132	113
R2	12	19	29	28	<1	6	<100	60
R3	13	19	31	29	1	9	<100	77
R4	13	19	32	30	<1	8	<100	127
R5	14	23	32	30	<1	11	<100	119

APPENDIX 2: TRACE ELEMENT LOADINGS